09/364,847 Search Strategy/Results

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     (FILE 'HOME' ENTERED AT 17:24:35 ON 14 FEB 2002)

     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     17:24:45 ON 14 FEB 2002
L1         50530 S POLYHYDROXYALKANOATE OR PHA
L2             1 S L1 AND (FUSION (W) ENZYME)
L3             0 S L1 AND ((BIFUNCTIONAL OR POLYFUNCTIONAL) (W) ENZYME)
L4           109 S L1 AND (FUSION (W) (PROTEIN OR POLYPEPTIDE OR GENE))
L5            67 S L4 NOT PY>1998
L6            22 DUP REM L5 (45 DUPLICATES REMOVED)
L7          2454 S POLYHYDROXYBUTYRATE
L8            12 S L7 AND (FUSION (W) (ENZYME OR PROTEIN OR POLYPEPTIDE OR GENE
L9             6 DUP REM L8 (6 DUPLICATES REMOVED)
L10           29 S KETOTHIOLASE AND FUSION
L11           12 DUP REM L10 (17 DUPLICATES REMOVED)
L12            9 S L11 NOT PY>1999
L13         3815 S POLYHYDROXYALKANOATE OR POLYHYDROXYBUTYRATE
L14            0 S L13 AND ((LINKER OR HINGE) (W) PEPTIDE)
L15           18 S L13 AND (LINKER OR HINGE)
L16            9 DUP REM L15 (9 DUPLICATES REMOVED)
L17            5 S L16 NOT PY>1999

=>
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L2 ANSWER 1 OF 1 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-195306 [17] WPIDS
DOC. NO. CPI: C2000-060596
TITLE: New enzymatic fusion proteins useful for producing polyhydroxyalkanoates in seeds of transgenic plants such as sunflower, soybean, and in bacteria, comprises enzymes involved in polyhydroxyalkanoates biosynthesis.
DERWENT CLASS: A23 C06 D16
INVENTOR(S): HUISMAN, G W; MADISON, L; PEOPLES, O P
PATENT ASSIGNEE(S): (META-N) METABOLIX INC
COUNTRY COUNT: 23
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000006747 | A2 | 20000210 | (200017)* | EN | 35 |
| RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE | | | | | |
| W: AU CA JP MX | | | | | |
| AU 9952502 | A | 20000221 | (200029) | | |
| EP 1100928 | A2 | 20010523 | (200130) | EN | |
| R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE | | | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000006747 | A2 | WO 1999-US17452 | 19990730 |
| AU 9952502 | A | AU 1999-52502 | 19990730 |
| EP 1100928 | A2 | EP 1999-937729 | 19990730 |
| | | WO 1999-US17452 | 19990730 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9952502 | A | Based on | WO 200006747 |
| EP 1100928 | A2 | Based on | WO 200006747 |

PRIORITY APPLN. INFO: US 1998-94674P 19980730
AN 2000-195306 [17] WPIDS
AB WO 200006747 A UPAB: 20000405
NOVELTY - A new fusion protein (I) comprises a heterodimer of poly((R)-3-hydroxyalkanoate) (**PHA**) biosynthetic enzymes fused through a linker.

DETAILED DESCRIPTION - (I) is of the formula: E1-Ln-E2 E2-Ln-E1

E1 and E2 = beta -ketothiolases, acyl-CoA reductases, **PHA** synthases, PHB (polyhydroxybutarate) synthetases, phasins, enoyl-CoA hydratases and beta -hydroxyacyl-ACP::coenzyme-A transferase;

Ln = a peptide of n amino acids that links E1 to E2 or E2 to E1.

INDEPENDENT CLAIMS are also included for the following:

(1) a plant expressing (I);

(2) a bacteria expressing (I); and

(3) a gene (II), encoding (I).

USE - The genetically engineered bacterial and plant systems are useful for enhanced production of PHAs in them. The fusion proteins can be expressed in transgenic microbial or plant crop **PHA** production systems. The fusions can be expressed in the cytosol or subcellular organelles of higher plant such as the seed of an oil crop Brassica, sunflower, soybean, corn, safflower, flax, palm or coconut and starch accumulating plants such as potato, tapioca, cassava, fiber plants such as cotton, hemp or the green tissue of tobacco, alfalfa, switch grass or other forage crops.

ADVANTAGE - Use of hybrid enzyme and its corresponding gene is advantageous since combining the two enzyme activities in a single transcriptional unit reduces the number of genes that need to be expressed in transgenic organisms, and the close proximity of two enzyme activities which catalyze sequential steps in a metabolic pathway. The **fusion enzyme** also allows for direct transfer of the reaction product from the first catalytic domain to the second domain.

DESCRIPTION OF DRAWING(S) - The figure shows the schematic construction of pTrcAB11 including phbA and phbB, on a single polypeptide with both thiolase and reductase activity.

Dwg.2/2

=> d his

09/364,847 Search Strategy/Results

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     (FILE 'HOME' ENTERED AT 17:24:35 ON 14 FEB 2002)

     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     17:24:45 ON 14 FEB 2002
L1         50530 S POLYHYDROXYALKANOATE OR PHA
L2             1 S L1 AND (FUSION (W) ENZYME)

=> s l1 and ((bifunctional or polyfunctional) (w) enzyme)
L3             0 L1 AND ((BIFUNCTIONAL OR POLYFUNCTIONAL) (W) ENZYME)

=> s l1 and (fusion (w) (protein or polypeptide or gene))
   5 FILES SEARCHED...
L4           109 L1 AND (FUSION (W) (PROTEIN OR POLYPEPTIDE OR GENE))

=> s l4 not py>1998
L5            67 L4 NOT PY>1998

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            22 DUP REM L5 (45 DUPLICATES REMOVED)

=> d 1- ibib abs
YOU HAVE REQUESTED DATA FROM 22 ANSWERS - CONTINUE? Y/(N):y

L6   ANSWER 1 OF 22     MEDLINE                          DUPLICATE 1
ACCESSION NUMBER:   1998349374     MEDLINE
DOCUMENT NUMBER:    98349374   PubMed ID: 9686585
TITLE:              Monocyte-dependent death of freshly isolated T lymphocytes:
                    induction by phorbolester and mitogens and differential
                    effects of catalase.
AUTHOR:             Wesch D; Marx S; Kabelitz D
CORPORATE SOURCE:   Department of Immunology, Paul-Ehrlich-Institute, Langen,
                    Germany.
SOURCE:             JOURNAL OF IMMUNOLOGY, (1998 Aug 1) 161 (3) 1248-56.
                    Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY:       United States
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH:        199808
ENTRY DATE:         Entered STN: 19980820
                    Last Updated on STN: 19980820
                    Entered Medline: 19980812
AB   Resting T cells are resistant to anti-Fas (CD95) mAb-mediated apoptosis
     but undergo apoptosis when triggered by anti-CD3 mAb or phorbolester PMA
     in the presence of PMA-activated monocytes. In this study, PMA, as well as
     the mitogens PHA and Con A, was found to induce death of resting
     T cells in the presence of autologous or allogeneic monocytes, while PWM
     was ineffective. Although several established monocytic and myelocytic
     cell lines were potent accessory cells for the mitogen-induced expansion
     of T lymphocytes, they all failed to replace plastic-adherent monocytes in
     the induction of monocyte-dependent cell death (MDCD) by PMA or
     PHA. CD45RA-positive cord blood T cells were as susceptible as
     peripheral blood T cells from adult donors to PMA-stimulated induction of
     MDCD. Using optimal concentrations of phorbolester, MDCD was inhibited
     neither by Fas-Fc fusion protein or neutralizing
     anti-Fas mAb, nor by inhibitors of IL-1beta-converting enzyme (ICE)-like
     proteases. In striking contrast, the H2O2 scavenger catalase completely
     prevented the PMA-stimulated T cell death, thereby revealing a potent
     mitogenic activity of PMA for human T cells in the presence of monocytes.
     Taken together, our results demonstrate that the accessory cell activity
     of monocytes/macrophages can be separated into "T cell death" and "T cell
     expansion" costimulatory functions, of which only the latter is mediated
     by established cell lines. Moreover, our results point to a pivotal role
     of reactive oxygen intermediates in the execution of MDCD triggered by
     PMA.

L6   ANSWER 2 OF 22     MEDLINE                          DUPLICATE 2
ACCESSION NUMBER:   1998118213     MEDLINE
DOCUMENT NUMBER:    98118213   PubMed ID: 9446659
TITLE:              Dendritic cells stimulate the expansion of bcr-abl specific
                    CD8+ T cells with cytotoxic activity against leukemic cells
                    from patients with chronic myeloid leukemia.
AUTHOR:             Nieda M; Nicol A; Kikuchi A; Kashiwase K; Taylor K; Suzuki
                    K; Tadokoro K; Juji T
CORPORATE SOURCE:   Japanese Red Cross Central Blood Center, Tokyo, Japan.
SOURCE:             BLOOD, (1998 Feb 1) 91 (3) 977-83.
```

Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 19980226
Entered Medline: 19980218

AB The role of T lymphocytes in the control of chronic myeloid leukemia (CML) after bone marrow transplantations has been clearly shown. This effect closely correlates with graft-versus-host disease (GVHD). A specific graft-versus-leukemia (GVL) effect separate from GVHD has been postulated but has been difficult to show. One possible target for specific GVL activity is the bcr-abl **fusion protein** characteristic of CML. We have investigated the use of normal peptide-pulsed dendritic cells for the generation of cytotoxic, bcr-abl-specific T cells from normal donors. T cells (CD3+, CD8+, TCR alpha beta+, and NK receptor-negative) generated from a normal donor (HLA A24, B52, B59, Cw1) after stimulation with autologous dendritic cells, primed with a 16 mer peptide spanning the b3a2 breakpoint of bcr-abl, lysed CML cells from the peripheral blood of seven patients with CML with the b3a2 breakpoint. CML cells from four patients with only the b2a2 breakpoint were not lysed. Phytohemagglutinin (**PHA**) blasts derived from peripheral blood of patients with CML were not lysed, suggesting that cytotoxicity was not due to alloreactivity. Blocking experiments with anti-HLA-A,B,C indicated that cytotoxicity was dependent on recognition of major histocompatibility complex (MHC) class I molecules, although cytotoxicity was not MHC-restricted because not all patients shared HLA types with the T-cell donor. Specificity for bcr-abl and absence of alloreactivity was confirmed by the presence of lytic activity against autologous and allogeneic class I HLA-A matched monocytes pulsed with the 16 mer bcr-abl fusion peptide, but not against unpulsed monocytes or monocytes pulsed with other peptides. These results show that bcr-abl-specific T cells with marked cytotoxic activity against CML cells can be generated and amplified from normal donor peripheral blood. Recognition of HLA molecules is essential for cytotoxicity but strict HLA identity is not required.

L6 ANSWER 3 OF 22 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1998256771 MEDLINE
DOCUMENT NUMBER: 98256771 PubMed ID: 9594415
TITLE: A recombinant fragment of Helicobacter pylori CagA affects proliferation of human cells.
AUTHOR: Rudnicka W; Covacci A; Wadstrom T; Chmiela M
CORPORATE SOURCE: Department of Infectious Biology, University of Lodz, Poland.
SOURCE: JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY, (1998 Mar) 49 (1) 111-9.
Journal code: A9B; 9114501. ISSN: 0867-5910.
PUB. COUNTRY: Poland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980717

AB The outcome of H. pylori infectins depends on proliferation of various host cells, including lymphocytes, monocytes and epithelial cells. In this study we showed that a recombinant **fusion protein** carrying an immunodominant region of H. pylori CagA antigen affected the proliferation of human cells. The rCagA inhibited **PHA**-driven T cell proliferation but enhanced the growth of epithelial HeLa cells, especially in the presence of granulocyte macrophage colony stimulating factor (GM-CSF). When THP-1 monocytes and Kato-3 epithelial cells from metastasis of gastric carcinoma were stimulated with GM-CSF, they were also susceptible to the inhibitory effect of rCagA. These results confirmed our earlier suggestion on the inhibition of T cell function by H. pylori CagA protein. However, antiproliferative activity of CagA antigen appears to be not restricted to T lymphocytes but modulatory effect of this protein seems to depend on the cell type.

L6 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:262155 CAPLUS
DOCUMENT NUMBER: 133:236567
TITLE: Establishment of stable transfectant cell lines producing human CTLA4Ig and CD28Ig fusion proteins
AUTHOR(S): Ryu, Yang-Seok; Chung, Yong-Hoon; Choi, Yong; Imm,

Dae-Cheul; Cho, Yang-Ja
CORPORATE SOURCE: Dep. Microbiology, Hanyang Univ., S. Korea
SOURCE: Hanyang Uidae Haksulchi (1998), 18(2), 101-118
CODEN: HIHAD3; ISSN: 0254-5942
PUBLISHER: Hanyang University, Medical College
DOCUMENT TYPE: Journal
LANGUAGE: Korean
AB CTLA-4, which is homologous to CD28, recognizes the same counter-receptors B7.1 and B7.2. Although initial researches implied that these mols. may act to each other synergistically, recent data suggest that CTLA-4 may acts as a neg. regulator of T cell activation. In this study human CD28 and CTLA-4 cDNAs were amplified from **PHA**-stimulated human peripheral blood lymphocytes mRNAs by using RT-PCR method. IgG1 Fc portion cDNA was amplified from human peripheral blood lymphocytes of patients with febrile illness and fused to C-terminal parts of CD28 and CTLA-4 extracellular domains. The fusion constructs were subcloned into pCI-neo vector and then transfected into Sp-2/0 murine myeloma cell. Stable cell lines were established by geneticin selection and cloning. In the sequence of CTLA-4 cDNA from 3 different healthy persons it was found that 2 sites of bases were different from that published in original paper and submitted to genbank database. The 49th and 331st bases of open reading frame were changed from adenine to guanine and from guanine to adenine resp. It was confirmed that the 17th and 111th amino acid of human CTLA-4 were alanine and threonine resp. 5 Different human CTLA-4 clones with serial N'-terminal deletion were constructed. CILA4/L was intact CTLA-4 gene of native form without any form of mutation. CTLA4/L2 was a mutant with deletion in gene coding N-terminal 6 amino acids. CTLA4/L3 was a mutant with deletion in gene coding N-terminal 11 amino acids. CTLA4/L4 was a mutant with deletion in gene coding N-terminal 16 amino acids. CTLA4/L5 was a mutant with deletion in gene coding N'-terminal 22 amino acids. Level of surface expression of CTLA4Ig on L1, L2, L3, L4 and L5 transfectant cell lines were 5%, 3%,-1.5%, 8%, and 6% resp. These 2 fusion proteins, CD28Ig and CTLA4Ig, would be useful tools for further researches on role of the costimulations in T cell activation.

L6 ANSWER 5 OF 22 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97333610 MEDLINE
DOCUMENT NUMBER: 97333610 PubMed ID: 9189770
TITLE: Construction and biological characterization of an interleukin-12 **fusion protein** (Flexi-12): delivery to acute myeloid leukemic blasts using adeno-associated virus.
AUTHOR: Anderson R; Macdonald I; Corbett T; Hacking G; Lowdell M W; Prentice H G
CORPORATE SOURCE: Department of Haematology, Royal Free Hospital School of Medicine, Hampstead, London, UK.
SOURCE: HUMAN GENE THERAPY, (1997 Jun 10) 8 (9) 1125-35.
Journal code: A12; 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970827
AB Interleukin-12 (IL-12) is a cytokine that exhibits pleiotropic effects on lymphocytes and natural killer cells and has been shown to have promise for the immunotherapy of cancer. The combination of the immune costimulatory molecule B7.1 and IL-12 has been shown to be synergistic for T cell activation. By transfecting tumor cells with both IL-12 and B7.1 cDNAs, it may be possible to use these modified targets as vaccines. A major obstacle in designing a vector to deliver these genes results from the structure of IL-12. Functional IL-12 is a heterodimer composed of two distinct subunits that are encoded by separate genes on different chromosomes. Production of functional IL-12 requires the coordinated expression of both genes. This presents several problems in vectors, particularly those in which additional genes, either a co-stimulatory gene or a selectable marker, are inserted. Therefore, we have constructed a single cDNA that encodes a single-chain protein, called Flexi-12, which retains all of the biological characteristics of recombinant IL-12 (rIL-12). The monomeric polypeptide Flexi-12 is able to induce the proliferation of phytohemagglutinin (**PHA**) blasts, induce **PHA** blasts to secrete interferon-gamma (IFN-gamma) and additionally, by preincubation, enhance the killing of K562 targets by PBLs. These phenomena are in a dose-dependent manner comparable to that seen with rIL-12. We have also shown that tyrosine phosphorylation of the STAT 4 transcription factor, which has been shown to be unique to the

IL-12 signaling pathway, occurs with Flexi-12 at levels similar to those seen with rIL-12. We have packaged Flexi-12 into a recombinant adeno-associated virus (AAV) and used this vector to infect acute myeloid leukemic (AML) blasts. Infected AML blasts produced between 2 and 6 ng of IL-12/10(6) cells per ml per 48 hr. These studies also confirm that AAV is an efficient delivery vehicle for cytokines to leukemic cells. Direct analysis of these modified cells acting as tumor vaccines is underway.

L6 ANSWER 6 OF 22 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998114594 MEDLINE
DOCUMENT NUMBER: 98114594 PubMed ID: 9446752
TITLE: Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis.
AUTHOR: Gambacorti-Passerini C; le Coutre P; Mologni L; Fanelli M; Bertazzoli C; Marchesi E; Di Nicola M; Biondi A; Corneo G M; Belotti D; Pogliani E; Lydon N B
CORPORATE SOURCE: Division of Experimental Oncology D and Medical Oncology C, Istituto Nazionale Tumori, Milan, Italy.. gambacorti@istitutotumori.mi.it
SOURCE: BLOOD CELLS, MOLECULES, AND DISEASES, (1997 Dec) 23 (3) 380-94.
Journal code: B5A; 9509932. ISSN: 1079-9796.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980407
Last Updated on STN: 19980407
Entered Medline: 19980324

AB The BCR/ABL **fusion protein** transforms myeloid stem cells. Both chronic myelogenous leukemias (CML) and a subset of acute lymphoblastic leukemias (ALL) are associated with the expression of BCR/ABL proteins. This knowledge has not yet been translated into any specific tool to control ABL driven neoplastic cells growth. CGP57148B is an ATP-competitive inhibitor of the ABL protein kinase; it has been shown to inhibit the kinase activity of ABL both in vitro and in vivo and to inhibit the growth of v-abl and bcr/abl transfectants, as well as the in vitro formation of bone marrow (BM)-derived colonies in the presence of growth factors in some CML patients. These studies were performed to investigate the activity of CGP57148B on the spontaneous proliferation of both fresh and cultured, leukemic and normal, BCR/ABL positive and negative cells, and to study its mechanism of action. Six cell lines derived from BCR/ABL+ leukemias (K562, BV173, KCL22, KU812, MC3, LAMA84), thirteen BCR/ABL negative lines, both neoplastic (KG1, SU-DHL-1, U937, Daudi, NB4, NB4.306) and derived from normal cells (**PHA** blasts, LAK, fibroblasts, LCL, renal epithelial cells, endothelial cells, CD34(+) cells), and 14 fresh leukemic samples were tested using a tritiated thymidine uptake assay. The in vivo phosphorylation of the BCR/ABL protein was evaluated by western blot, while apoptosis was detected by the annexin V/propidium binding test. The induction of differentiation was assayed by immunofluorescence using multiple antibodies. All six BCR/ABL+ lines showed a dose dependent inhibition of their spontaneous proliferative rate, which was not accompanied by differentiation. The treatment caused, within minutes, dephosphorylation of the BCR/ABL protein, followed in 16-24 hours by a decrease in cycling cells and induction of apoptosis. No significant inhibition of DNA synthesis was observed in any BCR/ABL negative normal or neoplastic line at concentrations </=3 microM, with the exception of fibroblasts and CD34 cells. Proliferation inhibition was observed also when using fresh samples obtained from two Ph+ ALL and 12 consecutive CML patients. Induction of apoptosis was observed in these samples too. The activity of CGP57148B can be monitored in ex vivo isolated or cultured cells using a simple and reproducible assay, without the need for exogenously added growth factors. This molecule possibly exerts its effects through the inhibition of the kinase activity of BCR/ABL and the subsequent initiation of apoptosis, without inducing cell differentiation. Some normal cells are also affected. These data support the use of CGP57148B in initial clinical studies; possible toxic effects on BM and fibroblast-derived cells will have to be closely monitored. The in vivo monitoring of patients will have to be focused on the induction of apoptosis in leukemic cells.


L6 ANSWER 7 OF 22 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97236316 MEDLINE
DOCUMENT NUMBER: 97236316 PubMed ID: 9125139
TITLE: The L1 adhesion molecule supports alpha v beta 3-mediated

migration of human tumor cells and activated T lymphocytes.
AUTHOR: Duczmal A; Schollhammer S; Katich S; Ebeling O; Schwartz-Albiez R; Altevogt P
CORPORATE SOURCE: Tumor Immunology Programme, German Cancer Research Center, Heidelberg, Germany.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1997 Mar 6) 232 (1) 236-9.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 19970506
Entered Medline: 19970422

AB The L1 adhesion molecule is a member of the immunoglobulin superfamily which is expressed by neural and hematopoietic cells. L1 is primarily a cell surface molecule but in its released form it becomes embedded in the extracellular matrix. In addition to the established L1-L1 homotypic interaction, L1 can bind to alpha v beta 3 in the human. The 6th Ig-like domain is critical for this function. We now demonstrate that a **fusion protein** containing the 6th Ig-like domain of L1 (6.L1-Fc) can support the migration of human MED-B1 (alpha v beta 3+) but not of Nalm-6 cells (alpha 5 beta 1+). The migration was blocked in the presence of a mab to alpha v beta 3 and was not seen on a 6.L1-Fc in which the RGD site was mutated. Activation of human T lymphocytes in the presence of **PHA** and PMA led to the induction of alpha v beta 3 and alpha v beta 5 expression and concomitantly induced migration of the cells on 6.L1-Fc. The migration was blocked by mabs to alpha v beta 3 but not to alpha v beta 5. Our results suggest that L1 exposed at the cell surface or as a matrix constituent can serve as a potent substrate for alpha v beta 3 mediated cell migration.

L6 ANSWER 8 OF 22 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96146527 MEDLINE
DOCUMENT NUMBER: 96146527 PubMed ID: 8550512
TITLE: Molecular analysis of the poly(3-hydroxyalkanoate) synthase gene from a methylotrophic bacterium, Paracoccus denitrificans.
AUTHOR: Ueda S; Yabutani T; Maehara A; Yamane T
CORPORATE SOURCE: Department of Bioproductive Sciences, Faculty of Agriculture, Utsunomiya University, Japan.
SOURCE: JOURNAL OF BACTERIOLOGY, (1996 Feb) 178 (3) 774-9.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D43764
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960306
Last Updated on STN: 19960306
Entered Medline: 19960222

AB A 3.6-kb EcoRI-SalI fragment of Paracoccus denitrificans DNA hybridized with a DNA probe carrying the poly(3-hydroxyalkanoate) (**PHA**) synthase gene (phaC) of Alcaligenes eutrophus. Nucleotide sequence analysis of this region showed the presence of a 1,872-bp open reading frame (ORF), which corresponded to a polypeptide with a molecular weight of 69,537. Upstream of the ORF, a promoter-like sequence was found. Escherichia coli carrying the **fusion gene** between lacZ and the ORF accumulated a level of poly(3-hydroxybutyrate) that was as much as 20 wt% of the cell dry weight in the presence of beta-ketothiolase and acetoacetylcoenzyme A reductase genes of A. eutrophus. The ORF was designated phaCPd. A plasmid vector carrying the phaCPd'-'lacZ **fusion gene** downstream of the promoter-like sequence expressed beta-galactosidase activity in P. denitrificans. When a multicopy and broad-host-range vector carrying the ORF along with the promoter-like sequence was introduced into P. denitrificans, the **PHA** content in the cells increased by twofold compared with cells carrying only a vector sequence.

L6 ANSWER 9 OF 22 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97136705 MEDLINE
DOCUMENT NUMBER: 97136705 PubMed ID: 8982082
TITLE: Cloning and characterization of a cDNA for rat CD30 homolog and chromosomal assignment of the genomic gene.
AUTHOR: Aizawa S; Satoh H; Horie R; Ito K; Choi S H; Takeuchi H;

Watanabe T
CORPORATE SOURCE: Department of Pathology, Institute of Medical Science, University of Tokyo, Japan.
SOURCE: GENE, (1996 Dec 5) 182 (1-2) 155-62.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D42117
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 20000303
Entered Medline: 19970122

AB CD30 is a member of the tumor necrosis factor receptor superfamily, which is expressed on some activated lymphocytes, virus-infected cells and transformed lymphocytes. To facilitate our understanding of biological functions and functional domains, we isolated rat cDNA clones encoding the rat homolog of human CD30 from a cDNA library of a rat T-cell line, TARL-2. The nucleotide sequence of the cDNA showed 73% homology with that of human CD30. The deduced rat CD30 protein consisted of 493 amino acids with an M(r) of 59 160 and contained a single transmembrane domain. It lacked the second repeat of the cysteine-rich motif in the extracellular domain found in human CD30. The amino acid sequence showed 51.8 and 61.2% identity with the cysteine-rich and the cytoplasmic domains, respectively. In the cytoplasmic domain, however, the amino acid sequence was highly conserved in about 100 residues near the C-terminus showing 77.7% identity, whereas the rest of the cytoplasmic domain showed 45.2% identity. This conservation suggests the functional importance of this region. Comparison with the recently reported mouse CD30 revealed 83.7% conservation of the amino acid sequence and a common structure of the extracellular domain which lacks the second cysteine-rich motif. Northern blots revealed a 3.4-kb mRNA in the **PHA**-activated spleen cells and human T-cell leukemia virus type 1 (HTLV-1)-infected rat T-cell lines, whereas smaller transcripts of 2.3 kb were found in the lung. A rabbit polyclonal antibody raised against GST-**fusion protein** of the cytoplasmic domain detected bands with an apparent M(r) of 80 kDa and 100- 110 kDa expressed in TARL-2 and spleen cells. Transient overexpression of rat CD30 in TARL-2 cells activated HIV LTR in a NF-kappa B site-dependent manner, indicating that CD30 signals activate NF-kappa B. The chromosomal location of the gene was identified by fluorescence in situ hybridisation at 5q36.2, and appeared to correspond to human 1p36, where human CD30 has been mapped. The identification and characterization of the rat counterpart of human CD30 will facilitate studies of the biological function of this molecule.

L6 ANSWER 10 OF 22 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 96181271 MEDLINE
DOCUMENT NUMBER: 96181271 PubMed ID: 8603537
TITLE: Defective B7 expression on antigen-presenting cells underlying T cell activation abnormalities in systemic lupus erythematosus (SLE) patients.
AUTHOR: Garcia-Cozar F J; Molina I J; Cuadrado M J; Marubayashi M; Pena J; Santamaria M
CORPORATE SOURCE: Unidad de Inmunologia, Hospital Universitario Reina Sofia, Spain.
SOURCE: CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1996 Apr) 104 (1) 72-9.
Journal code: DD7; 0057202. ISSN: 0009-9104.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19960524
Last Updated on STN: 19970203
Entered Medline: 19960515

AB Defective T cell functions, including IL-2 production and proliferation, have been shown in SLE patients. After T cell stimulation (first signal), a costimulatory signal (second signal) is required to achieve complete T cell activation. Main costimulatory signals are provided to T cells by B7 antigens (CD80 and CD86, expressed on antigen-presenting cells (APC)) upon interaction with its receptor, the CD28 molecule expressed on T cells. The aim of this study was to investigate the role of CD28/B7 interactions in the impaired T cell responses of SLE patients. We show that stimulation of T cells with phytohaemagglutinin (**PHA**) in the presence, but not in the absence, of anti-CD28 MoAb or B7+ cells results in tyrosine phosphorylation of specific substrates, transcription of mRNA and

production of IL-2 that is indistinguishable in SLE patients and healthy controls. Moreover, proliferation of costimulated T cells from SLE and controls was specifically abrogated by blocking the CD28/B7 interactions by means of addition to the culture of the CTLA4-Ig **fusion protein**. However, in most patients activated APC failed to up-regulate B7 molecules, giving rise to ineffective costimulatory signalling to T cells. These results indicate that the CD28/B7 costimulatory pathway is defective in SLE patients.

L6 ANSWER 11 OF 22 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 95247688 MEDLINE
DOCUMENT NUMBER: 95247688 PubMed ID: 7730285
TITLE: Identification of the region of a 14-kilodalton protein of Rhodococcus ruber that is responsible for the binding of this phasin to polyhydroxyalkanoic acid granules.
AUTHOR: Pieper-Furst U; Madkour M H; Mayer F; Steinbuchel A
CORPORATE SOURCE: Institut fur Mikrobiologie, Georg-August-Universitat Gottingen, Germany.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 May) 177 (9) 2513-23.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 19950608
Entered Medline: 19950601

AB The function of the polyhydroxyalkanoic acid (**PHA**) granule-associated GA14 protein of Rhodococcus ruber was investigated in Escherichia coli XL1-Blue, which coexpressed this protein with the polyhydroxybutyric acid (PHB) biosynthesis operon of Alcaligenes eutrophus. The GA14 protein had no influence on the biosynthesis rate of PHB in E. coli XL1-Blue(pSKCO7), but this recombinant E. coli strain formed smaller PHB granules than were formed by an E. coli strain that expressed only the PHB operon. Immunoelectron microscopy with GA14-specific antibodies demonstrated the binding of GA14 protein to these mini granules. In a previous study, two hydrophobic domains close to the C terminus of the GA14 protein were analyzed, and a working hypothesis that suggested an anchoring of the GA14 protein in the phospholipid monolayer surrounding the **PHA** granule core by these hydrophobic domains was developed (U. Pieper-Furst, M. H. Madkour, F. Mayer, and A. Steinbuchel, J. Bacteriol. 176:4328-4337, 1994). This hypothesis was confirmed by the construction of C-terminally truncated variants of the GA14 protein lacking the second or both hydrophobic domains and by the demonstration of their inability to bind to PHB granules. Further confirmation of the hypothesis was obtained by the construction of a **fusion protein** composed of the acetaldehyde dehydrogenase II of A. eutrophus and the C terminus of the GA14 protein containing both hydrophobic domains and by its affinity to native and artificial PHB granules.

L6 ANSWER 12 OF 22 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 95152045 MEDLINE
DOCUMENT NUMBER: 95152045 PubMed ID: 7849293
TITLE: ILA, the human 4-1BB homologue, is inducible in lymphoid and other cell lineages.
AUTHOR: Schwarz H; Valbracht J; Tuckwell J; von Kempis J; Lotz M
CORPORATE SOURCE: Sam and Rose Stein Institute for Research on Aging, San Diego, La Jolla, CA.
CONTRACT NUMBER: AR39799 (NIAMS)
CA51406 (NCI)
SOURCE: BLOOD, (1995 Feb 15) 85 (4) 1043-52.
Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19970203
Entered Medline: 19950316

AB We recently identified a gene that is induced by lymphocyte activation (ILA). The sequence of the full-length 1.4-kb cDNA characterized ILA as a new member of the nerve growth factor/tumor necrosis factor (NGF/TNF) receptor family and the human homologue of the murine T-cell-specific receptor 4-1BB. The present study demonstrates ILA mRNA isoforms at 4.4, 4.0, and 1.8 kb in poly-A+ RNA from activated, but not from resting human

peripheral blood T lymphocytes. A reverse transcriptase-polymerase chain reaction (RT-PCR) assay was used to study tissue distribution and regulation of ILA expression. The gene was induced in T lymphocytes by phytohemagglutinin (**PHA**), phorbol myristate acetate (PMA), and antibody to CD3, in B lymphocytes by PMA and antibodies to cell surface Ig, and in blood monocytes by interleukin-1 beta (IL-1 beta), lipopolysaccharide (LPS), and PMA. In T lymphocytes, ILA mRNA was detectable 1.5 hours after stimulation, reached maximal levels at 8 hours, and declined to background levels by 48 hours. Induction of ILA mRNA required protein synthesis and was primarily due to increased transcription. Actinomycin D reduced ILA mRNA levels in activated lymphocytes 50% within 30 minutes, demonstrating a relatively short half-life of this mRNA. Analysis of nonlymphoid cells showed that ILA mRNA was not detectable in resting cells. However, in contrast to the lymphoid-specific expression of the murine 4-1BB gene, ILA was detected in nonlymphoid cells, including epithelial and hepatoma cells after stimulation with IL-1 beta. ILA was not detectable in several brain derived cell lines. The ILA cDNA encodes a 30-kD protein as demonstrated by in vitro translation, and this protein is immunoprecipitated by antisera that were raised against ILA peptides or a glutathione-S-transferase **fusion protein**. Flow cytometry showed expression of ILA protein on a subset of activated T or B lymphocytes. In conclusion, activation-dependent expression of ILA is found not only in T lymphocytes, but also in B lymphocytes, monocytes, and diverse nonlymphoid cell types.

L6 ANSWER 13 OF 22 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 96111968 MEDLINE
DOCUMENT NUMBER: 96111968 PubMed ID: 8777726
TITLE: Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor.
AUTHOR: Yao Z; Fanslow W C; Seldin M F; Rousseau A M; Painter S L; Comeau M R; Cohen J I; Spriggs M K
CORPORATE SOURCE: Immunex Corporation, Seattle, Washington 98101, USA.
CONTRACT NUMBER: AR41053 (NIAMS)
HG00734 (NHGRI)
SOURCE: IMMUNITY, (1995 Dec) 3 (6) 811-21.
Journal code: CCF; 9432918. ISSN: 1074-7613.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U31993
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960924
Last Updated on STN: 19990129
Entered Medline: 19960916

AB Herpesvirus Saimiri gene 13 (HVS13) exhibits 57% identity with the predicted sequence of a T cell-derived molecule termed CTLA8. Recombinant HVS13 and CTLA8 stimulate transcriptional factor NF-kappa B activity and interleukin-6 (IL-6) secretion in fibroblasts, and costimulate T cell proliferation. An HVS13.Fc **fusion protein** was used to isolate a cDNA encoding a novel receptor that also binds CTLA8. This receptor is unrelated to previously identified cytokine receptor families. A recombinant soluble receptor inhibited T cell proliferation and IL-2 production induced by **PHA**, concanavalin A (conA), and anti-TCR MAb. These results define CTLA8 and HVS13 as novel cytokines that bind to a novel cytokine receptor. We propose to call these molecules IL-17, vIL-17, and IL-17R, respectively.

L6 ANSWER 14 OF 22 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 95230679 MEDLINE
DOCUMENT NUMBER: 95230679 PubMed ID: 7714894
TITLE: Exclusive expression of C. elegans osm-3 kinesin gene in chemosensory neurons open to the external environment.
AUTHOR: Tabish M; Siddiqui Z K; Nishikawa K; Siddiqui S S
CORPORATE SOURCE: Dept of Ecological Engineering, Toyohashi University of Technology, Japan.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 Mar 31) 247 (3) 377-89.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D38632
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950524
Last Updated on STN: 19950524

Entered Medline: 19950515

AB In Caenorhabditis elegans three genetic loci osm-3, unc-104 and unc-116 have been identified, which encode anterograde motor kinesin. Here we show that osm-3 encodes a 672 amino acid long kinesin-like protein (KLP) that contains all three functional domains similar to the kinesin heavy chain, including a globular motor region, an alpha-helical coiled-coil rod, and a globular tail region. OSM-3 shows homology in both the motor and rod domains with kinesins from divergent species such as mouse KIF3, and sea urchin KRP95, and also with the rod domains of several non-kinesin proteins, such as myosin, ezrin, outer membrane proteins alpha precursor OMPA, yeast intracellular protein transport USO1, and the rat neurofilament NF-H. Temporal and spatial expression of the osm-3::lacZ **fusion gene** during development is limited to an exclusive set of 26 chemosensory neurons whose dendritic endings are exposed to the external environment, including six IL2 neurons of the inner labial sensilla, eight pairs of amphid neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK) in the head, and two pairs of phasmid neurons ( **PHA** and PHB) in the tail. Our data are consistent with the known structural defects in the amphid and phasmid sensilla in osm-3 mutants and also show the expression of the gene in IL2 neurons. Temporally, the gene is differentially expressed in all three types of chemosensory sensilla. Further work on osm-3, unc-104 and unc-116 mutants should give insight into the in vivo functions of the kinesin family during C. elegans neurogenesis.

L6 ANSWER 15 OF 22 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:737237 CAPLUS
DOCUMENT NUMBER: 126:85366
TITLE: Cloning and characterization of a cDNA for rat CD30 homolog and chromosomal assignment of the genomic gene
AUTHOR(S): Aizawa, Shigemi; Satoh, Hitoshi; Horie, Ryouichi; Ito, Kinji; Choi, Soon Hee; Takeuchi, Hajime; Watanabe, Toshiki
CORPORATE SOURCE: Department of Pathology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108, Japan
SOURCE: Gene (1995), Volume Date 1996, 182(1/2), 155-162
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CD30 is a member of the tumor necrosis factor receptor superfamily, which is expressed on some activated lymphocytes, virus-infected cells and transformed lymphocytes. To facilitate our understanding of biol. functions and functional domains, we isolated rat cDNA clones encoding the rat homolog of human CD30 from a cDNA library of a rat T-cell line, TARL-2. The nucleotide sequence of the cDNA showed 73% homol. with that of human CD30. The deduced rat CD30 protein consisted of 493 amino acids with an Mr of 59160 and contained a single transmembrane domain. It lacked the second repeat of the cysteine-rich motif in the extracellular domain found in human CD30. The amino acid sequence showed 51.8 and 61.2% identity with the cysteine-rich and the cytoplasmic domains, resp. In the cytoplasmic domain, however, the amino acid sequence was highly conserved in about 100 residues near the C-terminus showing 77.7% identity, whereas the rest of the cytoplasmic domain showed 45.2% identity. This conservation suggests the functional importance of this region. Comparison with the recently reported mouse CD30 revealed 83.7% conservation of the amino acid sequence and a common structure of the extracellular domain which lacks the second cysteine-rich motif. Northern blots revealed a 3.4-kb mRNA in the **PHA**-activated spleen cells and human T-cell leukemia virus type 1 (HTLV-1)-infected rat T-cell lines, whereas smaller transcripts of 2.3kb were found in the lung. A rabbit polyclonal antibody raised against GST-**fusion protein** of the cytoplasmic domain detected bands with an apparent Mr of 80kDa and 100-110kDa expressed in TARL-2 and spleen cells. Transient overexpression of rat CD30 in TARL-2 cells activated HIV LTR in a NF-.kappa.B site-dependent manner, indicating that CD30 signals activate NF-.kappa.B. The chromosomal location of the gene was identified by fluorescence in situ hybridization at 5q36.2, and appeared to correspond to human 1p36, where human CD30 has been mapped. The identification and characterization of the rat counterpart of human CD30 will facilitate studies of the biol. function of this mol.

L6 ANSWER 16 OF 22 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 93305286 MEDLINE
DOCUMENT NUMBER: 93305286 PubMed ID: 7763712
TITLE: Cloning and characterization of the Methylobacterium extorquens polyhydroxyalkanoic-acid-synthase structural

gene.
AUTHOR: Valentin H E; Steinbuchel A
CORPORATE SOURCE: Institut fur Mikrobiologie, Georg-August-Universitat Gottingen, Germany.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1993 Jun) 39 (3) 309-17.
Journal code: AMC; 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
OTHER SOURCE: GENBANK-L07893
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 19960129
Entered Medline: 19930730

AB A cosmid gene bank of partially EcoRI-digested genomic DNA from Methylobacterium extorquens IBT no. 6 was screened for DNA fragments restoring polyhydroxyalkanoic-acid (**PHA**) accumulation in the **PHA**-negative mutant Alkaligenes eutrophus H16 PHB-4. The M. extorquens **PHA**-synthase structural gene phaCMex was mapped on a 23-kbp EcoRI fragment by complementation studies, by hybridization experiments with heterologous DNA probes from A. eutrophus H16 encoding for phaA, phaB and phaC and by nucleic acid sequence analysis. Evidence for the presence of genes for a beta-ketothiolase or an acetoacetyl-coenzyme A reductase on this fragment was not obtained. The nucleotide sequence of a 3.7-kbp region was obtained. It contained the entire 1.815-kbp phaCMex plus approximately each 900-bp upstream and downstream of phaCMex.PhaCMex encoded a protein of 605 amino acids with a relative molecular mass (M(r)) of 66742, which exhibited 38.1% amino acid identity with the A. eutrophus **PHA** synthase. Determination of the N-terminal amino acid sequence of an M(r) 65,000 protein, which was enriched concomitantly with the purification of **PHA** granules in sucrose gradients, revealed a sequence that was identical with the amino acid sequence deduced from the most probable translation start codon except for a valine, which was obviously removed post-translationally. Enzyme analysis, which was done with the native gene and a phaCMex'-'lacZ **fusion gene**, gave no evidence for expression of phaCMex in Escherichia coli.

L6 ANSWER 17 OF 22 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 93169322 MEDLINE
DOCUMENT NUMBER: 93169322 PubMed ID: 8435662
TITLE: T cell and NK cell mediated graft-versus-leukaemia reactivity following donor buffy coat transfusion to treat relapse after marrow transplantation for chronic myeloid leukaemia.
AUTHOR: Jiang Y Z; Cullis J O; Kanfer E J; Goldman J M; Barrett A J
CORPORATE SOURCE: Department of Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.
SOURCE: BONE MARROW TRANSPLANTATION, (1993 Feb) 11 (2) 133-8.
Journal code: BON; 8702459. ISSN: 0268-3369.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19930402
Entered Medline: 19930325

AB Two patients with chronic myeloid leukaemia in cytogenetic relapse following T lymphocyte-depleted BMT were treated with transfusions of donor buffy coat leucocytes. In both patients the marrow reverted to a completely normal karyotype and was negative for the BCR-ABL **fusion gene** transcript by polymerase chain reaction analysis. Before buffy coat transfusion the cytotoxic T lymphocyte precursor frequency against pre-BMT patient leukaemia cells (Lk-CTLP) was lower than that against pre-BMT patient **PHA**-transformed lymphocytes (Ly-CTLP) in both cases. At 2 weeks (case 1) and 8 weeks (case 2) after transfusion this ratio inverted so that Lk-CTLP predominated. Natural killer (NK) function fell initially and then recovered to exceed pre-transfusion values prior to normalization of the bone marrow karyotype. These changes in cytotoxic T lymphocytes and NK cells following donor buffy coat transfusions for patients with relapsed chronic myeloid leukaemia after marrow transplantation support the concept of a graft-versus-leukaemia effect mediated by both MHC restricted and non-restricted pathways.

L6 ANSWER 18 OF 22 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 93160296 MEDLINE
DOCUMENT NUMBER: 93160296 PubMed ID: 8431514
TITLE: Affinity purification and characterization of anti-Tac(Fv)-C3-PE38KDEL: A highly potent cytotoxic agent specific to cells bearing IL-2 receptors.
AUTHOR: Spence C; Nachman M; Gately M K; Kreitman R J; Pastan I; Bailon P
CORPORATE SOURCE: Protein Biochemistry Department, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110.
SOURCE: BIOCONJUGATE CHEMISTRY, (1993 Jan-Feb) 4 (1) 63-8. Journal code: A1T; 9010319. ISSN: 1043-1802.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19930402
Entered Medline: 19930317

AB A chimeric, single chain antibody fused immunotoxin, denoted anti-Tac(Fv)-C3-PE38KDEL, was engineered and expressed in Escherichia coli. The microbially expressed anti-Tac(Fv)-C3-PE38KDEL was solubilized from inclusion bodies using guanidine hydrochloride, and subsequently refolded in a redox buffer via thiol/disulfide exchange. The recombinant immunotoxin from the crude extract was purified employing receptor-affinity chromatography, which is based upon biological function and involved the immobilized p55 subunit of human IL-2 receptor. The cytotoxic activity of this immunotoxin was measured by the IL-2 dependent phytohemagglutinin (**PHA**) blast proliferation inhibition and HUT-102 protein synthesis inhibition assays, in which the IC50 values were 41.5 and 0.8 pM, respectively. The biochemical homogeneity and authenticity of the purified material were determined by gel permeation chromatography, amino acid composition and N-terminal sequence analyses, SDS-PAGE, isoelectric focusing, and Western blotting. The receptor-affinity-purified immunotoxin was shown to be highly effective in specifically killing cells bearing IL-2 receptors. Anti-Tac(Fv)-C3-PE38KDEL is a powerful immunosuppressant which may be a potentially useful therapeutic agent in the prevention of allograft rejection and in the treatment of autoimmune diseases. Another anticipated application of this **fusion protein** is as a chemotoxin in the treatment of some forms of cancer.

L6 ANSWER 19 OF 22 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 92325473 MEDLINE
DOCUMENT NUMBER: 92325473 PubMed ID: 1624793
TITLE: Cloning and expression of recombinant Aspergillus fumigatus allergen I/a (rAsp f I/a) with IgE binding and type I skin test activity.
AUTHOR: Moser M; Crameri R; Menz G; Schneider T; Dudler T; Virchow C; Gmachl M; Blaser K; Suter M
CORPORATE SOURCE: Swiss Institute of Allergy and Asthma Research (SIAF), Davos.
SOURCE: JOURNAL OF IMMUNOLOGY, (1992 Jul 15) 149 (2) 454-60. Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920821
Last Updated on STN: 19920821
Entered Medline: 19920811

AB Aspergillus fumigatus secretes an 18-kDa nonglycosylated IgE-binding protein. This protein was previously shown to be a ribotoxin, like alpha-sarcin and mitogillin. A 686-bp long A. fumigatus cDNA encoding an 18-kDa ribotoxin was cloned and expressed in Escherichia coli as a **fusion protein** with six adjacent histidines (rAsp f I/a). rAsp f I/a was purified to homogeneity by Ni(2+)-chelate affinity chromatography and refolded. The recombinant protein was enzymatically active resulting in the cleavage of 28S rRNA within a universally conserved region. rAsp f I/a was cytotoxic for EBV immortalized or **PHA** stimulated human PBMC. Furthermore, rAsp f I/a was recognized by murine mAb made against an 18-kDa ribotoxin. IgE of individuals allergic to A. fumigatus bound to rAsp f I/a as shown by ELISA, dot blots, and Western blots. rAsp f I/a elicited positive immediate type I skin reactions in individuals allergic to A. fumigatus but not in healthy control individuals. The results show that rAsp f I/a has similar

functional characteristics when compared to the native 18-kDa ribotoxin. rAsp f I/a expressed in E. coli can therefore be used as a standardized Ag/allergen for serologic and clinical diagnosis of A. fumigatus-associated diseases.

L6 ANSWER 20 OF 22 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 92011688 MEDLINE
DOCUMENT NUMBER: 92011688 PubMed ID: 1655771
TITLE: Cyclosporin A blocks calcium-dependent pathways of gene activation.
AUTHOR: Baldari C T; Macchia G; Heguy A; Melli M; Telford J L
CORPORATE SOURCE: Department of Evolutionary Biology, University of Siena, Italy.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Oct 5) 266 (28) 19103-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199111
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19970203
Entered Medline: 19911108

AB We have used an interleukin-2 (IL-2) promoter-CAT **fusion gene** to study activation of IL-2 gene expression by IL-1, phytohemagglutinin (**PHA**), phorbol myristate acetate (PMA), and calcium ionophore in the murine thymoma line EL4 and the human lymphoma line Jurkat. The two cell lines respond differently to combinations of these stimuli. IL-1 in combination with suboptimal concentration of PMA induced chloramphenicol acetyltransferase (CAT) activity in EL4. In Jurkat cells, IL-1 failed to synergize with PMA or **PHA**. Cotransfection with the IL-2/CAT gene and a construct capable of expressing murine T-cell type IL-1 receptors converted Jurkat cells to IL-1 responsiveness. IL-1 in combination with **PHA** but not with PMA resulted in induction of CAT activity in these cells. Induction of IL-2/CAT activity by all stimuli in both cell lines was blocked by the presence of EGTA in the culture medium. EGTA did not inhibit IL-1/PMA activation of an SV40 early promoter-CAT **fusion gene** in either EL4 or Jurkat cells; therefore, calcium was not required for IL-1 or PMA signal transduction. Jurkat cells were shown to differ from EL4 in their requirement for calcium mobilization. Two different calcium-dependent pathways of gene activation were distinguished, both of which were blocked by the immunosuppressive drug cyclosporin A.

L6 ANSWER 21 OF 22 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:606100 CAPLUS
DOCUMENT NUMBER: 115:206100
TITLE: Genetic and molecular analysis of the Alcaligenes eutrophus poly(hydroxyalkanoate)-biosynthetic genes and accumulation of **PHA** in recombinant bacteria
AUTHOR(S): Steinbuchel, Alexander; Schubert, Peter; Timm, Arnulf; Pries, Andreas
CORPORATE SOURCE: Inst. Mikrobiol., Georg-August-Univ., Goettingen, D-3400, Fed. Rep. Ger.
SOURCE: NATO ASI Ser., Ser. E (1990), 186(Novel Biodegrad. Microb. Polym.), 143-59
CODEN: NAESDI
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mol. methods were applied to analyze the A. eutrophus PHB biosynthetic pathway. The genes for .beta.-ketothiolase (phbA), NADPH-dependent acetoacetyl-CoA reductase (phbB), and PHB synthase (phbC) are presumably organized in 1 operon with phbC being the 1st gene transcribed. The exact beginning of phbC was identified by the anal. of the amino acid sequence of a phbC'-lacZ'-**fusion protein**. The promoter (TTGACA-18N-AACAAT) was identified 307 bp 5'-upstream of phbC by the anal. of clones harboring promoter deletions and by nuclease protection assay. Octanoate-grown cells of a recombinant strain of Pseudomonas oleovorans harboring the A. eutrophus PHB biosynthetic genes accumulated a blend of the homopolyester PHB and of the copolyester P(3HO-co-3HHx). The MW-values for these polymers were 2.96 .times. 106 and 0.35 .times. 106, resp. Cells of 16 strains of P. aeruginosa and of 9 other pseudomonads belonging to rRNA homol. group I accumulated a polymer consisting of mainly 3-hydroxydecanoate from simple carbohydrates such as gluconate. The gene locus which upon insertional inactivation by Tn5::mob results in the phenotype PHB-leaky was identified and characterized.

L6 ANSWER 22 OF 22 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:193221 CAPLUS
DOCUMENT NUMBER: 112:193221
TITLE: Rinderpest virus vaccine using recombinant vaccina virus
INVENTOR(S): Yamanouchi, Kazuya; Yoshikawa, Yasuhiro; Tsukiyama, Kyoko; Asano, Kaori; Maruyama, Tadashi; Sugimoto, Masanobu
PATENT ASSIGNEE(S): Toa Nenryo Kogyo K. K., Japan
SOURCE: Eur. Pat. Appl., 48 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 330781 | A1 | 19890906 | EP 1988-310203 | 19881031 |
| EP 330781 | B1 | 19960306 | | |
| R: CH, DE, FR, GB, LI | | | | |
| JP 01218590 | A2 | 19890831 | JP 1988-44134 | 19880229 |
| CA 1313151 | A1 | 19930126 | CA 1988-579958 | 19881012 |

PRIORITY APPLN. INFO.: JP 1988-44134 19880229
AB Live rinderpest virus vaccines are prepd. by expression of cloned genes for antigenic rinderpest virus proteins, i.e. hemagglutinin (HA) and viral cell-**fusion protein**, using vaccinia virus vectors in the vaccinated host. CDNA's for these genes were cloned using heterologous probes and placed under the control of a no. of suitable promoters and these expression cassettes were introduced into vaccinia virus vectors by homologous recombination. Rabbits vaccinated with 1 .times. 108 plaque forming units of HA expression vector showed a short-term rise in temp. but no other ill effects and produced anti-HA antibody at titers of 26-27 within two weeks. Subsequent infection of these rabbits with 103 IC50 of rinderpest virus elicited no symptoms of infection whereas control animals died 13 days after infection with the same dose.

=> s polyhydroxybutyrate
L7 2454 POLYHYDROXYBUTYRATE

=> s l7 and (fusion (w) (enzyme or protein or polypeptide or gene))
5 FILES SEARCHED...
L8 12 L7 AND (FUSION (W) (ENZYME OR PROTEIN OR POLYPEPTIDE OR GENE))

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9 6 DUP REM L8 (6 DUPLICATES REMOVED)

=> d 1- ibib abs
YOU HAVE REQUESTED DATA FROM 6 ANSWERS - CONTINUE? Y/(N):y

L9 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:207445 CAPLUS
DOCUMENT NUMBER: 135:328605
TITLE: Identification of the intracellular polyhydroxyalkanoate depolymerase gene of Paracoccus denitrificans and some properties of the gene product
AUTHOR(S): Gao, D.; Maehara, A.; Yamane, T.; Ueda, S.
CORPORATE SOURCE: Laboratory of Applied Microbiology, Department of Bioproductive Science, Faculty of Agriculture, Utsunomiya University, Utsunomiya, 321-8505, Japan
SOURCE: FEMS Microbiol. Lett. (2001), 196(2), 159-164
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Paracoccus denitrificans degraded poly(3-hydroxybutyrate) (PHB) in the cells under carbon source starvation. Intracellular poly(3-hydroxyalkanoate) (PHA) depolymerase gene (phaZ) was identified near the PHA synthase gene (phaC) of P. denitrificans. Cell ext. of Escherichia coli carrying lacZ-phaZ **fusion gene** degraded protease-treated PHB granules. Reaction products were thought to be mainly D(-)-3-hydroxybutyrate (3HB) dimer and 3HB oligomer. Diisopropylfluorophosphonate and Triton X-100 exhibited an inhibitory effect on the degrdn. of PHB granules. When cell ext. of the recombinant

E. coli was used, Mg2+ ion inhibited PHB degrdn. However, the inhibitory effect by Mg2+ ion was not obsd. using the cell ext. of P. denitrificans.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 6 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-532624 [48] WPIDS
DOC. NO. CPI: C2000-158593
TITLE: New nucleic acid fragment encoding proteins involved in polyhydroxyalkanoate (PHA) biosynthesis, useful in the production of transgenic plants or recombinant plant cells which can express PHAs such as **polyhydroxybutyrate**.
DERWENT CLASS: A11 C06 D16
INVENTOR(S): CANNON, F C; CANNON, M C; GRUYS, K J; MCCOOL, G J; VALENTIN, H E
PATENT ASSIGNEE(S): (UYMA-N) UNIV MASSACHUSETTS
COUNTRY COUNT: 90
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000040730 | A1 | 20000713 | (200048)* | EN | 148 |

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

| | | | | | |
|---|---|---|---|---|---|
| AU 2000024949 | A | 20000724 | (200052) | | |
| EP 1141317 | A1 | 20011010 | (200167) | EN | |

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000040730 | A1 | WO 2000-US364 | 20000107 |
| AU 2000024949 | A | AU 2000-24949 | 20000107 |
| EP 1141317 | A1 | EP 2000-903161 | 20000107 |
| | | WO 2000-US364 | 20000107 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 2000024949 | A | Based on | WO 200040730 |
| EP 1141317 | A1 | Based on | WO 200040730 |

PRIORITY APPLN. INFO: US 1999-115592P 19990107
AN 2000-532624 [48] WPIDS
AB WO 200040730 A UPAB: 20001001
NOVELTY - A nucleic acid fragment (N1) encoding proteins involved in polyhydroxyalkanoate (PHA) biosynthesis, is new. N1, a 7916 base pair (bp) sequence (S1) defined in the specification, was isolated from Bacillus megaterium and it contains nine open reading frames.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid segment (N2) comprising a sequence encoding a PHA inclusion body associated protein (PIBAP), where the nucleic acid sequence is selected from:

(a) a nucleic acid sequence which is 80% or totally identical to the 510 (I), 438 (II), 504 (III) or 1086 (IX) bp sequence (all sequences defined in the specification);

(b) a nucleic acid sequence that hybridizes under stringent conditions to (I), (II), (III) or (IX), or their complements;

(c) a nucleic acid sequence encoding a protein which is 80% or totally identical to the 170 (IV), 146 (V), 168 (VI) or 362 (X) amino acid sequence (all sequences defined in the specification); or

(d) a nucleic acid sequence encoding a protein that is immunoreactive with an antibody prepared using (IV), (V), (VI) or (X) as an antigen;

(2) a nucleic acid segment (N2) comprising a nucleic acid sequence encoding a 3-keto-acyl-CoA reductase protein, where the nucleic acid sequence is selected from:

(a) a nucleic acid sequence which is 80% or totally identical to the 741 bp sequence (VII);

(b) a nucleic acid sequence that hybridizes under stringent

conditions to (VII) or its complement;
(c) a nucleic acid sequence encoding a protein which is 80% or totally identical to the 247 amino acid sequence (VIII); and
(d) a nucleic acid sequence encoding a protein that is immunoreactive with an antibody prepared using (VII) as an antigen;
(3) an isolated PIBAP comprising an amino acid sequence selected from:
(a) an amino acid sequence which is 80% or totally identical to (IV), (V), (VI) or (X); or
(b) an amino acid sequence that is immunoreactive with an antibody prepared using (IV), (V), (VI) or (X) as an antigen;
(4) an isolated 3-keto-acyl-CoA reductase protein comprising an amino acid sequence selected from:
(a) an amino acid sequence which is 80% or totally identical to (VIII); or
(b) an amino acid sequence that is immunoreactive with an antibody prepared using (VIII) as an antigen;
(5) a recombinant vector comprising in the 5' to 3' direction:
(a) a promoter that directs transcription of a structural nucleic acid sequence encoding a PIBAP or 3-keto-acyl-CoA reductase;
(b) a structural nucleic acid selected from N1 or N2;
(c) a 3' transcription terminator;
(6) a recombinant host cell comprising a nucleic acid segment from N1 or N2;
(7) a genetically transformed plant or its cell comprising a nucleic acid containing in the 5' to 3' direction:
(a) a promoter that directs transcription of a structural nucleic acid sequence encoding a PIBAP or 3-keto-acyl-CoA reductase;
(b) a structural nucleic acid sequence selected from N1 or N2;
(c) a 3' transcription terminator; and
(d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid sequence;
(8) a method of preparing host cells useful to produce a PIBAP or 3-keto-acyl-CoA reductase, comprising selecting a host cell and transforming it with a nucleic acid sequence selected from N1 or N2, respectively;
(9) a method of preparing plants useful to produce a PIBAP or 3-keto-acyl-CoA reductase, comprising:
(a) selecting a host plant cell;
(b) transforming the selected host plant cell with a recombinant vector having a structural nucleic acid sequence selected from N1 or N2;
(c) obtaining transformed host plant cells; and
(d) regenerating the transformed host plant cells;
(10) a fusion protein comprising a green fluorescent protein subunit and a PIBAP or 3-keto-acyl-CoA reductase;
(11) a nucleic acid segment encoding the fusion protein of (10);
(12) a method of preparing PHA, comprising culturing a cell or growing a plant containing a nucleic acid sequence encoding a 3-keto-acyl-CoA reductase protein and a nucleic acid sequence encoding a PHA synthase protein; and
(13) a method of preparing PHA, comprising:
(a) obtaining a recombinant host cell comprising nucleic acid sequences encoding beta -ketothiolase protein, 3-keto-acyl-CoA reductase protein, PHA synthase protein, beta -hydroxyacyl-CoA dehydrase and acyl-CoA dehydrogenase or an enoyl-CoA reductase; and
(b) culturing the cell under conditions suitable for the preparation of PHA.
ACTIVITY - None given.
MECHANISM OF ACTION - None given.
No biological data is provided.
USE - The nucleic acids are useful for creating transgenic plants or recombinant plant cells which have the capability of expressing PHAs such as polyhydroxybutyrate, polyhydroxyvalerate, polyhydroxyhexanoate, polyhydroxyoctanoate, polyhydroxydecanoate or their copolymers.
Dwg.0/10

L9 ANSWER 3 OF 6 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-204620 [18] WPIDS
DOC. NO. CPI: C2000-063036
TITLE: Rhodococcus ruber gene encoding polyhydroxyalkanoate (PHA) polymer granule-associated protein GA14, useful for controlling polymer granule size and number in PHA-producing bacteria.
DERWENT CLASS: A23 A96 B04 D16
INVENTOR(S): PIEPER-FURST, U; STEINBUECHEL, A
PATENT ASSIGNEE(S): (MONS) MONSANTO CO
COUNTRY COUNT: 1

09/364,847 Search Strategy/Results

PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| US 6022729 | A | 20000208 | (200018)* | | 32 |

APPLICATION DETAILS:

| PATENT NO | KIND | | APPLICATION | DATE |
|---|---|---|---|---|
| US 6022729 | A | Cont of | US 1995-500735 | 19950711 |
| | | Cont of | US 1996-598175 | 19960207 |
| | | | US 1996-702870 | 19960826 |

PRIORITY APPLN. INFO: GB 1994-14506 19940718
AN 2000-204620 [18] WPIDS
AB US 6022729 A UPAB: 20000412
NOVELTY - A gene isolated from Rhodococcus ruber encoding the polyhydroxyalkanoate (PHA) polymer granule-associated protein GA14 is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated DNA molecule encoding a PHA polymer granule-associated protein, comprising a 1.0 kilobase (kb) XhoI-ApaI genomic restriction fragment from R. ruber, where the restriction fragment has a defined 80 base pair sequence given in the specification;

(2) a method for controlling polymer granule size and number in a PHA-producing bacterium, comprising insertion of a gene encoding R. ruber GA14 protein into the genome of the bacterium;

(3) a method for binding a desired protein to a PHA granule in a bacterium that produces the granule, comprising inserting into the genome of the bacterium a DNA molecule that encodes the desired protein and also encodes the HD1 and/or HD2 domain of the R. ruber GA14 protein;

(4) a bacterium transformed with genes encoding enzymes necessary for catalyzing PHA production linked to regulatory sequences for controlling expression of the genes and a gene or **fusion gene** encoding the R. ruber GA14 protein.

USE - The gene can be inserted into the genome of PHA-producing bacteria to control polymer granule size and number (claimed) (smaller granules may be produced in higher yields; larger granules may be easier to isolate). Fragments of the gene encoding the C-terminal hydrophobic domains HD1 and/or HD2 of the GA14 protein can be fused to a protein-encoding sequence and used to enhance binding of the protein to polymer granules in PHA-producing bacteria (claimed). PHAs, including **polyhydroxybutyrate**, poly-3-hydroxyvalerate and poly-3-hydroxyoctanoate, are biocompatible, biodegradable, thermoplastic polymers useful e.g. for packaging and medical application.

Dwg.0/7

L9 ANSWER 4 OF 6 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999335124 MEDLINE
DOCUMENT NUMBER: 99335124 PubMed ID: 10408639
TITLE: Substrate and binding specificities of bacterial **polyhydroxybutyrate** depolymerases.
AUTHOR: Kasuya K; Ohura T; Masuda K; Doi Y
CORPORATE SOURCE: Polymer Chemistry Laboratory, The Institute of Physical and Chemical Research (RIKEN), Saitama, Japan.
SOURCE: INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, (1999 May) 24 (4) 329-36.
Journal code: AY6; 7909578. ISSN: 0141-8130.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990910
Last Updated on STN: 19990910
Entered Medline: 19990824

AB The substrate specificities of three extracellular **polyhydroxybutyrate** (PHB) depolymerases from Alcaligenes faecalis (PhaZ Afa), Pseudomonas stutzeri (PhaZ Pst), and Comamonas acidovorans (PhaZ Cac), which are grouped into types A and B based on the position of a lipase box sequence in the catalytic domain, were examined for films of 12 different aliphatic polyesters. Each of these PHB depolymerases used was capable of hydrolyzing poly(3-hydroxybutyrate) (P(3HB)), poly(3-hydroxypropionate) (P(3HP)), poly(4-hydroxybutyrate) (P(4HB)), poly(ethylene succinate) (PESU), and poly(ethylene adipate) (PEA) but could not hydrolyze another seven polyesters. In addition, the binding

characteristics of substrate binding domains from PhaZ Afa, PhaZ Cac, and PHB depolymerase from Comamonas testosteroni (PhaZ Cte) were studied by using fusions with glutathione S-transferase (GST). All of fusion proteins adsorbed strongly on the surfaces of polyester granules of P(3HB), P(3HP), and poly(2-hydroxypropionate) (P(2HP)) which was not hydrolyzed by the PHB depolymerases used in this study, while they did not bind on Avicel and chitin granules. The adsorption kinetics of the fusion proteins to the surface of P(3HB) and P(2HP) granules were found to obey the Langmuir isotherm. The cross-area per molecule of **fusion protein** bound to P(3HB) granules was estimated to be 12+/-4 nm2/molecule. It has been suggested that the active sites in catalytic domains of PHB depolymerases have a similar conformational structure, and that several amino acids in substrate-binding domains of PHB depolymerases interact specifically with the surface of polyesters.

L9 ANSWER 5 OF 6 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998069478 MEDLINE
DOCUMENT NUMBER: 98069478 PubMed ID: 9406404
TITLE: Biochemical and molecular characterization of the **polyhydroxybutyrate** depolymerase of Comamonas acidovorans YM1609, isolated from freshwater.
AUTHOR: Kasuya K; Inoue Y; Tanaka T; Akehata T; Iwata T; Fukui T; Doi Y
CORPORATE SOURCE: Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Kanagawa, Japan.
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Dec) 63 (12) 4844-52.
Journal code: 6K6; 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB003186
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 20000303
Entered Medline: 19980213

AB Comamonas acidovorans YM1609 secreted a **polyhydroxybutyrate** (PHB) depolymerase into the culture supernatant when it was cultivated on poly(3-hydroxybutyrate) [P(3HB)] or poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] as the sole carbon source. The PHB depolymerase was purified from culture supernatant of C. acidovorans by two chromatographic methods, and its molecular mass was determined as 45,000 Da by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme was stable at temperatures below 37 degrees C and at pH values of 6 to 10, and its activity was inhibited by diisopropyl fluorophosphonate. The liquid chromatography analysis of water-soluble products revealed that the primary product of enzymatic hydrolysis of P(3HB) was a dimer of 3-hydroxybutyric acid. Kinetics of enzymatic hydrolysis of P(3HB) film were studied. In addition, a gene encoding the PHB depolymerase was cloned from the C. acidovorans genomic library. The nucleotide sequence of this gene was found to encode a protein of 494 amino acids (M(r), 51,018 Da). Furthermore, by analysis of the N-terminal amino acid sequence of the purified enzyme, the molecular mass of the mature enzyme was calculated to be 48,628 Da. Analysis of the deduced amino acid sequence suggested a domain structure of the protein containing a catalytic domain, fibronectin type III module as linker, and a putative substrate-binding domain. Electron microscopic visualization of the mixture of P(3HB) single crystals and a **fusion protein** of putative substrate-binding domain with glutathione S-transferase demonstrated that the **fusion protein** adsorbed strongly and homogeneously to the surfaces of P(3HB) single crystals.

L9 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:56525 CAPLUS
DOCUMENT NUMBER: 114:56525
TITLE: Molecular analysis of the Alcaligenes eutrophus poly(3-hydroxybutyrate) biosynthetic operon: identification of the N terminus of poly(3-hydroxybutyrate) synthase and identification of the promoter
AUTHOR(S): Schubert, Peter; Krueger, Niels; Steinbuechel, Alexander
CORPORATE SOURCE: Inst. Mikrobiol., Georg-August-Univ., Goettingen, D-3400, Fed. Rep. Ger.
SOURCE: J. Bacteriol. (1991), 173(1), 168-75
CODEN: JOBAAY; ISSN: 0021-9193

09/364,847 Search Strategy/Results

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mol. methods have been applied to analyze the expression of the A. eutrophus poly(3-hydroxybutyrate) (PHB) synthase gene (phbC). The translational initiation codon was identified by anal. of the amino acid sequence of a PHB synthase-.beta.-galactosidase **fusion protein**. This protein was purified to almost gel electrophoretic homogeneity by chromatog. on DEAE-Sephacel and on aminophenyl-.beta.-D-thiogalactopyranoside-Sepharose from cells of A. eutrophus which harbored a phbC'-'lacZ **fusion gene**. A sequence (TTGACA-18N-AACAAT), exhibiting homol. to the Escherichia coli .sigma.70 promoter consensus sequence, was identified approx. 310 bp 5' upstream from the translation initiation codon. An S1 nuclease protection assay mapped the transcription start point of phbC 6 bp downstream from this promoter. The location of the promoter was confirmed by analyzing the expression of active PHB synthase in clones of E. coli harboring 5' upstream deletions of phbC ligated to the promoter of the lacZ gene (lacZp) in a Bluescript vector. Plasmids do181 and do218, which were deleted for the 1st 108 or 300 bp of the phbC structural gene, resp., conferred the ability to synthesize large amts. of different truncated PHB synthase proteins to the cells. These proteins contributed to approx. 10% of the total cellular protein as estd. from sodium dodecyl sulfate-polyacrylamide gels. The modified PHB synthase encoded by plasmid do181 was still active. Clones in which the lacZp-'phbC fusion harbored the complete phbC structural gene plus the phbC ribosome binding site did not overexpress PHB synthase.

=> s ketothiolase and fusion
L10 29 KETOTHIOLASE AND FUSION

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11 12 DUP REM L10 (17 DUPLICATES REMOVED)

=> s l11 not py>1999
L12 9 L11 NOT PY>1999

=> d 1- ibib abs
YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L12 ANSWER 1 OF 9 MEDLINE
ACCESSION NUMBER: 2000174435 MEDLINE
DOCUMENT NUMBER: 20174435 PubMed ID: 10709652
TITLE: Studies on regulation of the peroxisomal beta-oxidation at the 3-**ketothiolase** step. Dissection of the rat liver thiolase B gene promoter.
AUTHOR: Latruffe N; Nicolas-Frances V; Dasari V K; Osumi T
CORPORATE SOURCE: University of Burgundy, LBMC, Faculty Gabriel, Dijon, France.. latruffe@u-bourgogne.fr
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1999) 466 253-9.
Journal code: 2LU; 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000421
Last Updated on STN: 20000421
Entered Medline: 20000411
AB The peroxisomal 3-oxoacyl-CoA thiolase (thiolase) is the last enzyme involved in the beta-oxidation of fatty acids. The enzyme cleaves long chain fatty acyl-CoA to generate acetyl-CoA and shortened acyl-CoA. The enzyme is nuclear encoded, synthesized in the cytoplasm and transported into peroxisomes. The thiolase B gene is inducible by the peroxisome proliferator compounds, like other genes involved in beta-oxidation of fatty acids in peroxisomes. The importance of studying thiolase is that it generates acetyl-CoA which is the precursor for the synthesis of molecules like cholesterol and fatty acids. The structural and functional analysis of thiolase at molecular level may add to the knowledge of fatty acid metabolism and further the obesity phenomenon. It is known that several genes mediate lipid homeostasis in target organs like liver, adipose tissue and are regulated by peroxisome proliferator activated receptors (PPAR alpha and PPAR gamma). To elucidate the mechanism of induction of rat liver thiolase B gene, an upstream 2.8 kb fragment containing promoter element has been subcloned and partially sequenced. The sequence analysis revealed a putative PPRE (Peroxisome Proliferator Response Element) of

AGACCT T TGAACC sequence at -681 to -668 [Kliever et al. (1992) Nature 358:771-774]. By transient expression of a luciferase reporter gene in HeLa cells, we conclude that the identified PPRE could be functional in induction of thiolase B gene, but other sequences of genes might be involved.

L12 ANSWER 2 OF 9 MEDLINE
ACCESSION NUMBER: 1999069318 MEDLINE
DOCUMENT NUMBER: 99069318 PubMed ID: 9851987
TITLE: Cloning and molecular analysis of the Poly(3-hydroxybutyrate) and Poly(3-hydroxybutyrate-co-3-hydroxyalkanoate) biosynthesis genes in Pseudomonas sp. strain 61-3.
AUTHOR: Matsusaki H; Manji S; Taguchi K; Kato M; Fukui T; Doi Y
CORPORATE SOURCE: Polymer Chemistry Laboratory and the RIKEN Group of Japan Science and Technology Corporation, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan.
SOURCE: JOURNAL OF BACTERIOLOGY, (1998 Dec) 180 (24) 6459-67. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB014757; GENBANK-AB014758
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 19990209
Entered Medline: 19990128

AB Two types of polyhydroxyalkanoate (PHA) biosynthesis gene loci (phb and pha) of Pseudomonas sp. strain 61-3, which produces a blend of poly(3-hydroxybutyrate) [P(3HB)] homopolymer and a random copolymer poly(3-hydroxybutyrate-co-3-hydroxyalkanoate) [P(3HB-co-3HA] consisting of 3HA units of 4 to 12 carbon atoms, were cloned and analyzed at the molecular level. In the phb locus, three open reading frames encoding polyhydroxybutyrate (PHB) synthase (PhbCPs), beta-**ketothiolase** (PhbAPs), and NADPH-dependent acetoacetyl coenzyme A reductase (PhbBPs) were found. The genetic organization showed a putative promoter region, followed by phbBPs-phbAPs-phbCPs. Upstream from phbBPs was found the phbRPs gene, which exhibits significant similarity to members of the AraC/XylS family of transcriptional activators. The phbRPs gene was found to be transcribed in the opposite direction from the three structural genes. Cloning of phbRPs in a relatively high-copy vector in Pseudomonas sp. strain 61-3 elevated the levels of beta-galactosidase activity from a transcriptional phb promoter-lacZ **fusion** and also enhanced the 3HB fraction in the polyesters synthesized by this strain, suggesting that PhbRPs is a positive regulatory protein controlling the transcription of phbBACPs in this bacterium. In the pha locus, two genes encoding PHA synthases (PhaC1Ps and PhaC2Ps) were flanked by a PHA depolymerase gene (phaZPs), and two adjacent open reading frames (ORF1 and phaDPs), and the gene order was ORF1, phaC1Ps, phaZPs, phaC2Ps, and phaDPs. Heterologous expression of the cloned fragments in PHA-negative mutants of Pseudomonas putida and Ralstonia eutropha revealed that PHB synthase and two PHA synthases of Pseudomonas sp. strain 61-3 were specific for short chain length and both short and medium chain length 3HA units, respectively.

L12 ANSWER 3 OF 9 MEDLINE
ACCESSION NUMBER: 96146527 MEDLINE
DOCUMENT NUMBER: 96146527 PubMed ID: 8550512
TITLE: Molecular analysis of the poly(3-hydroxyalkanoate) synthase gene from a methylotrophic bacterium, Paracoccus denitrificans.
AUTHOR: Ueda S; Yabutani T; Maehara A; Yamane T
CORPORATE SOURCE: Department of Bioproductive Sciences, Faculty of Agriculture, Utsunomiya University, Japan.
SOURCE: JOURNAL OF BACTERIOLOGY, (1996 Feb) 178 (3) 774-9. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D43764
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960306
Last Updated on STN: 19960306
Entered Medline: 19960222

AB A 3.6-kb EcoRI-SalI fragment of Paracoccus denitrificans DNA hybridized with a DNA probe carrying the poly(3-hydroxyalkanoate) (PHA) synthase gene

(phaC) of Alcaligenes eutrophus. Nucleotide sequence analysis of this region showed the presence of a 1,872-bp open reading frame (ORF), which corresponded to a polypeptide with a molecular weight of 69,537. Upstream of the ORF, a promoter-like sequence was found. Escherichia coli carrying the **fusion** gene between lacZ and the ORF accumulated a level of poly(3-hydroxybutyrate) that was as much as 20 wt% of the cell dry weight in the presence of beta-**ketothiolase** and acetoacetylcoenzyme A reductase genes of A. eutrophus. The ORF was designated phaCPd. A plasmid vector carrying the phaCPd'-'lacZ **fusion** gene downstream of the promoter-like sequence expressed beta-galactosidase activity in P. denitrificans. When a multicopy and broad-host-range vector carrying the ORF along with the promoter-like sequence was introduced into P. denitrificans, the PHA content in the cells increased by twofold compared with cells carrying only a vector sequence.

L12 ANSWER 4 OF 9 MEDLINE
ACCESSION NUMBER: 95362679 MEDLINE
DOCUMENT NUMBER: 95362679 PubMed ID: 7635832
TITLE: Phosphate concentration regulates transcription of the Acinetobacter polyhydroxyalkanoic acid biosynthetic genes.
AUTHOR: Schembri M A; Bayly R C; Davies J K
CORPORATE SOURCE: Department of Microbiology, Monash University, Clayton, Victoria, Australia.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Aug) 177 (15) 4501-7. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L37761
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19980206
Entered Medline: 19950913

AB The polyhydroxyalkanoic acid (PHA) biosynthetic gene locus was cloned and characterized from an Acinetobacter sp. isolated from activated sludge. Nucleotide sequence analysis identified three clustered genes, phaAAc (encoding a beta-**ketothiolase**), phaBAc (encoding an acetoacetyl coenzyme A reductase), and phaCAc (encoding a PHA synthase). In addition, an open reading frame (ORF1) with potential to encode a 13-kDa protein was identified within this locus. The sequence of the putative translational product of ORF1 does not show significant similarity to any sequences in the database. A plasmid containing the Acinetobacter pha locus conferred the ability to accumulate poly-beta-hydroxybutyrate on its Escherichia coli host. These genes appear to lie in an operon transcribed by two promoters upstream of phaBAc, an apparent constitutive promoter, and a second promoter induced by phosphate starvation and under pho regulon control. These as well as a number of additional potential transcription start points were identified by a combination of primer extension and promoter-chloramphenicol acetyltransferase gene **fusion** studies carried out in Acinetobacter or E. coli transformants.

L12 ANSWER 5 OF 9 MEDLINE
ACCESSION NUMBER: 95330674 MEDLINE
DOCUMENT NUMBER: 95330674 PubMed ID: 7606665
TITLE: High-level poly(beta-hydroxybutyrate) production in recombinant Escherichia coli in sugar-free, complex medium.
AUTHOR: Kalousek S; Lubitz W
CORPORATE SOURCE: Institute of Microbiology and Genetics, University of Vienna, Austria.
SOURCE: CANADIAN JOURNAL OF MICROBIOLOGY, (1995) 41 Suppl 1 216-21. Journal code: CJ3; 0372707. ISSN: 0008-4166.
PUB. COUNTRY: Canada
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950828
Last Updated on STN: 19980206
Entered Medline: 19950811

AB The poly(beta-hydroxybutyrate) (PHB) biosynthetic genes of Alcaligenes eutrophus that are organized in a single operon (phbCAB) have been cloned in Escherichia coli, where the expression of the genes in the wild-type phb operon from plasmid p4A leads to the formation of 10 or 50-80% PHB/cell dry mass when the cells are grown in Luria-Bertani medium alone or supplemented with 1% glucose (w/v), respectively. To further stimulate PHB formation independent of additional carbon source in Luria-Bertani medium, molecular methods have been applied to provide efficient E. coli

transcription and translation signals for the PHB synthase gene (phbC). The lac promoter present upstream of the phbC sequence allows its expression to be controlled depending on the LacI status of the chosen host strain. The T7 gene 10 ribosome binding site is utilized for translational initiation. PHB production in E. coli was compared in strains either harboring plasmid p4A containing the intact phbCAB operon or harboring two compatible plasmids carrying the beta-**ketothiolase** (phbA) and acetoacetyl-CoA-reductase (phbB) genes under transcriptional control of the lac promoter-operator region and also carrying separately the phbC gene with its natural promoter sequence. In addition, plasmid pSYN allowing the phbC gene to be expressed under new transcription and translation conditions combined with plasmid pUMS gave rise to the same amount of PHB formation (70% PHB cell dry mass) in E. coli when grown in Luria-Bertani medium without glucose supplement.

L12 ANSWER 6 OF 9 MEDLINE
ACCESSION NUMBER: 93305286 MEDLINE
DOCUMENT NUMBER: 93305286 PubMed ID: 7763712
TITLE: Cloning and characterization of the Methylobacterium extorquens polyhydroxyalkanoic-acid-synthase structural gene.
AUTHOR: Valentin H E; Steinbuchel A
CORPORATE SOURCE: Institut fur Mikrobiologie, Georg-August-Universitat Gottingen, Germany.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1993 Jun) 39 (3) 309-17.
Journal code: AMC; 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
OTHER SOURCE: GENBANK-L07893
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 19960129
Entered Medline: 19930730

AB A cosmid gene bank of partially EcoRI-digested genomic DNA from Methylobacterium extorquens IBT no. 6 was screened for DNA fragments restoring polyhydroxyalkanoic-acid (PHA) accumulation in the PHA-negative mutant Alkaligenes eutrophus H16 PHB-4. The M. extorquens PHA-synthase structural gene phaCMex was mapped on a 23-kbp EcoRI fragment by complementation studies, by hybridization experiments with heterologous DNA probes from A. eutrophus H16 encoding for phaA, phaB and phaC and by nucleic acid sequence analysis. Evidence for the presence of genes for a beta-**ketothiolase** or an acetoacetyl-coenzyme A reductase on this fragment was not obtained. The nucleotide sequence of a 3.7-kbp region was obtained. It contained the entire 1.815-kbp phaCMex plus approximately each 900-bp upstream and downstream of phaCMex.PhaCMex encoded a protein of 605 amino acids with a relative molecular mass (M(r)) of 66742, which exhibited 38.1% amino acid identity with the A. eutrophus PHA synthase. Determination of the N-terminal amino acid sequence of an M(r) 65,000 protein, which was enriched concomitantly with the purification of PHA granules in sucrose gradients, revealed a sequence that was identical with the amino acid sequence deduced from the most probable translation start codon except for a valine, which was obviously removed post-translationally. Enzyme analysis, which was done with the native gene and a phaCMex'-'lacZ **fusion** gene, gave no evidence for expression of phaCMex in Escherichia coli.

L12 ANSWER 7 OF 9 MEDLINE
ACCESSION NUMBER: 83282749 MEDLINE
DOCUMENT NUMBER: 83282749 PubMed ID: 6882421
TITLE: Characterization of enzymatic deficiencies of branched chain amino-acid catabolism in human fibroblasts by genetic complementation.
AUTHOR: Coude F X; Grimber G; Parvy P; Pham Dinh D; Bardet J; Saudubray J M
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1983 Jul 18) 114 (1) 175-82.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198309
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 20000303
Entered Medline: 19830909

AB Leucine and Isoleucine metabolism in cultured skin fibroblasts from patients with leucinosis, beta-**Ketothiolase** deficiency, propionic, methylmalonic and isovaleric acidemia, was compared with that in normal fibroblasts. A simple assay was developed using (U14C) Isoleucine and (U14C) Leucine as substrates. Radioactive incorporation into protein aminoacids were measured. The (U14C) branched chain aminoacid incorporation into proteins provides an estimation of the protein synthesis and the incorporation ratio (14C) Aspartate + (14C) Glutamate/(14C) branched chain aminoacid, measures the integrity of the metabolic pathway. Complementation tests permits to characterize the genetic defect. The incorporation ratio was significantly decreased in blocked pathways, namely in leucinosis and isovaleric acidemia in the presence of (U14C) Leucine and in Leucinosis, beta-**Ketothiolase** deficiency, propionic and methylmalonic acidemia in the presence of (U14C) Isoleucine. There was a significant restoration of activity in mutant strains with distinct genetic defects after polyethylene-glycol **fusion**. This assay provides a valuable tool to screen for enzymatic deficiencies of branched chain aminoacid catabolism.

L12 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:606100 CAPLUS
DOCUMENT NUMBER: 115:206100
TITLE: Genetic and molecular analysis of the Alcaligenes eutrophus poly(hydroxyalkanoate)-biosynthetic genes and accumulation of PHA in recombinant bacteria
AUTHOR(S): Steinbuchel, Alexander; Schubert, Peter; Timm, Arnulf; Pries, Andreas
CORPORATE SOURCE: Inst. Mikrobiol., Georg-August-Univ., Goettingen, D-3400, Fed. Rep. Ger.
SOURCE: NATO ASI Ser., Ser. E (1990), 186(Novel Biodegrad. Microb. Polym.), 143-59
CODEN: NAESDI
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mol. methods were applied to analyze the A. eutrophus PHB biosynthetic pathway. The genes for .beta.-**ketothiolase** (phbA), NADPH-dependent acetoacetyl-CoA reductase (phbB), and PHB synthase (phbC) are presumably organized in 1 operon with phbC being the 1st gene transcribed. The exact beginning of phbC was identified by the anal. of the amino acid sequence of a phbC'-lacZ'-**fusion** protein. The promoter (TTGACA-18N-AACAAT) was identified 307 bp 5'-upstream of phbC by the anal. of clones harboring promoter deletions and by nuclease protection assay. Octanoate-grown cells of a recombinant strain of Pseudomonas oleovorans harboring the A. eutrophus PHB biosynthetic genes accumulated a blend of the homopolyester PHB and of the copolyester P(3HO-co-3HHx). The MW-values for these polymers were 2.96 .times. 106 and 0.35 .times. 106, resp. Cells of 16 strains of P. aeruginosa and of 9 other pseudomonads belonging to rRNA homol. group I accumulated a polymer consisting of mainly 3-hydroxydecanoate from simple carbohydrates such as gluconate. The gene locus which upon insertional inactivation by Tn5::mob results in the phenotype PHB-leaky was identified and characterized.

L12 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:528661 CAPLUS
DOCUMENT NUMBER: 115:128661
TITLE: Molecular analysis of the Alcaligenes eutrophus PHB-biosynthetic genes: identification of the NH2-terminus of PHB synthase and identification of the transcription start site of phbC
AUTHOR(S): Schubert, Peter; Pries, Andreas; Krueger, Niels; Steinbuechel, Alexander
CORPORATE SOURCE: Inst. Mikrobiol., Georg-August-Univ., Goettingen, D-3400, Fed. Rep. Ger.
SOURCE: NATO ASI Ser., Ser. E (1990), 186(Novel Biodegrad. Microb. Polym.), 447-8
CODEN: NAESDI
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 12.5-kb EcoRI DNA-fragment PP1 isolated from genomic DNA of A. eutrophus H16, which encodes the genes for .beta.-**ketothiolase** (phbA), NADPH-dependent acetoacetyl-CoA reductase (phbB), and PHB synthase (phbC) has been cloned recently. The entire structural gene phbC was cloned in both orientations into the multicopy vector Bluescript KS-. The resulting hybrid plasmids were referred to as pSAP33 and pSAP37. Nested deletions of both plasmids were generated using the exonuclease III/mung bean nuclease system. The deleted plasmids were used for sequence anal. of phbC. Six relevant clones, harboring pSAP33 with a deleted region upstream of the phbC-region, were analyzed for PHB synthase activity in

order to localize the promoter region. The translation start site was identified by NH2-terminal sequence anal. of a phbC'-lacZ' **fusion** protein. A signal for the first methionine was not obtained, probably because this amino acid is removed by posttranslational modification. To det. the correct start point of transcription, a nuclease protection assay was used for hybridization and for nuclease S1 reaction. The transcription start point was detected 307 nucleotides upstream of the structural gene in both assays.

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=> s polyhydroxyalkanoate or polyhydroxybutyrate
L13       3815 POLYHYDROXYALKANOATE OR POLYHYDROXYBUTYRATE

=> s l13 and ((linker or hinge) (w) peptide)
L14          0 L13 AND ((LINKER OR HINGE) (W) PEPTIDE)

=> s l13 and (linker or hinge)
L15         18 L13 AND (LINKER OR HINGE)

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16           9 DUP REM L15 (9 DUPLICATES REMOVED)

=> s l16 not py>1999
L17           5 L16 NOT PY>1999

=> d 1- ibib abs
YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L17  ANSWER 1 OF 5     MEDLINE
ACCESSION NUMBER:    1999091373     MEDLINE
DOCUMENT NUMBER:     99091373   PubMed ID: 9872779
TITLE:               Cloning and characterization of the
                     polyhydroxybutyrate depolymerase gene of
                     Pseudomonas stutzeri and analysis of the function of
                     substrate-binding domains.
AUTHOR:              Ohura T; Kasuya K I; Doi Y
CORPORATE SOURCE:    Graduate School of Science and Engineering, Saitama
                     University, Shimo-ohkubo, Urawa-shi, Saitama 338-8570,
                     Japan.
SOURCE:              APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1999 Jan) 65 (1)
                     189-97.
                     Journal code: 6K6; 7605801. ISSN: 0099-2240.
PUB. COUNTRY:        United States
                     Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
OTHER SOURCE:        GENBANK-AB012225
ENTRY MONTH:         199902
ENTRY DATE:          Entered STN: 19990301
                     Last Updated on STN: 19990301
                     Entered Medline: 19990216
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AB The extracellular **polyhydroxybutyrate** (PHB) depolymerase gene (phaZPst) of Pseudomonas stutzeri was cloned and sequenced. phaZPst was composed of 1,728 bp encoding a protein of 576 amino acids. Analyses of the N-terminal amino acid sequence and the matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrum of the purified enzyme showed that the mature enzyme consisted of 538 amino acids with a deduced molecular mass of 57,506 Da. Analysis of the deduced amino acid sequence of the protein revealed a domain structure containing a catalytic domain, putative **linker** region, and two putative substrate-binding domains (SBDI and SBDII). The putative **linker** region was similar to the repeating units of the cadherin-like domain of chitinase A from Vibrio harveyi and chitinase B from Clostridium paraputrificum. The binding characteristics of SBDs to poly([R]-3-hydroxybutyrate) [P(3HB)] and chitin granules were characterized by using fusion proteins of SBDs with glutathione S-transferase (GST). These GST fusion proteins with SBDII and SBDI showed binding activity toward P(3HB) granules but did not bind on chitin granules. It has been suggested that the SBDs of the depolymerase interact specifically with the surface of P(3HB). In addition, a kinetic analysis for the enzymatic hydrolysis of 3-hydroxybutyrate oligomers of various sizes has suggested that the catalytic domain of the enzyme recognizes at least two monomeric units as substrates.

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L17  ANSWER 2 OF 5     MEDLINE
ACCESSION NUMBER:    1998069478     MEDLINE
DOCUMENT NUMBER:     98069478   PubMed ID: 9406404
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TITLE: Biochemical and molecular characterization of the **polyhydroxybutyrate** depolymerase of Comamonas acidovorans YM1609, isolated from freshwater.
AUTHOR: Kasuya K; Inoue Y; Tanaka T; Akehata T; Iwata T; Fukui T; Doi Y
CORPORATE SOURCE: Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Kanagawa, Japan.
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Dec) 63 (12) 4844-52.
Journal code: 6K6; 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB003186
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 20000303
Entered Medline: 19980213

AB Comamonas acidovorans YM1609 secreted a **polyhydroxybutyrate** (PHB) depolymerase into the culture supernatant when it was cultivated on poly(3-hydroxybutyrate) [P(3HB)] or poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] as the sole carbon source. The PHB depolymerase was purified from culture supernatant of C. acidovorans by two chromatographic methods, and its molecular mass was determined as 45,000 Da by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme was stable at temperatures below 37 degrees C and at pH values of 6 to 10, and its activity was inhibited by diisopropyl fluorophosphonate. The liquid chromatography analysis of water-soluble products revealed that the primary product of enzymatic hydrolysis of P(3HB) was a dimer of 3-hydroxybutyric acid. Kinetics of enzymatic hydrolysis of P(3HB) film were studied. In addition, a gene encoding the PHB depolymerase was cloned from the C. acidovorans genomic library. The nucleotide sequence of this gene was found to encode a protein of 494 amino acids (M(r), 51,018 Da). Furthermore, by analysis of the N-terminal amino acid sequence of the purified enzyme, the molecular mass of the mature enzyme was calculated to be 48,628 Da. Analysis of the deduced amino acid sequence suggested a domain structure of the protein containing a catalytic domain, fibronectin type III module as **linker**, and a putative substrate-binding domain. Electron microscopic visualization of the mixture of P(3HB) single crystals and a fusion protein of putative substrate-binding domain with glutathione S-transferase demonstrated that the fusion protein adsorbed strongly and homogeneously to the surfaces of P(3HB) single crystals.

L17 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:49682 CAPLUS
DOCUMENT NUMBER: 130:168751
TITLE: Synthesis of amino acid-, carbohydrate-, coumarin-, and biotin-labeled oligo[(R)-3-hydroxybutanoic acids] (OHB)
AUTHOR(S): Fritz, Monica G.; Seebach, Dieter
CORPORATE SOURCE: Laboratorium Organische Chemie, Eidgenoessische Technische Hochschule Zurich, Zurich, CH-8092, Switz.
SOURCE: Helv. Chim. Acta (1998), 81(12), 2414-2429
CODEN: HCACAV; ISSN: 0018-019X
PUBLISHER: Verlag Helvetica Chimica Acta AG
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The benzyl esters of oligo[(R)-3-hydroxybutanoic acids] (OHB) contg. 2, 16, or 32 HB units were coupled at the hydroxy terminus with arginine (by esterification with carbodiimide), with glucose (by acetalization with glucosyl trichloroacetimidate), and with 7-(dimethylamino)coumarin-4-acetic acid and biotin (by amide formation through a glycine **linker**) to give, after deprotection(s), the corresponding "labeled" OHB acids. The resp. novel 16- and 32mer derivs. exhibit distinct water soly. (table) or may be detected (in minute amts.) by fluorescence spectroscopy, properties required for biochem. investigations.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:700915 CAPLUS
DOCUMENT NUMBER: 128:1355
TITLE: Structural and functional characteristics of poly(3-hydroxybutyric acid) depolymerases

AUTHOR(S): Shinomiya, Miho; Doi, Yoshiharu
CORPORATE SOURCE: Polymer Chem. Lab., RIKEN, Japan
SOURCE: RIKEN Rev. (1997), 15, 27-28
CODEN: RIREE6; ISSN: 0919-3405
PUBLISHER: Institute of Physical and Chemical Research
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 5 refs. Several bacteria secrete poly(3-hydroxybutyric acid) (PHB) depolymerases to hydrolyze water-insol. poly[(R)-3-hydroxybutyric acid] (P[(R)-3HB]) in various environments. These enzymes consist of 4 domains, signal peptide, catalytic domain, **linker** region, and substrate-binding domain. The active site is a Ser residue in a lipase box pentapeptide Gly-X1-Ser-X2-Gly which is common for serine hydrolases. Hydrolysis of solid P[(R)-3HB] by PHB depolymerase proceeds by a 2-step reaction, namely adsorption and hydrolysis. The substrate-binding domain has the ability to adhere to solid P[(R)-3HB] by itself.

L17 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:256386 CAPLUS
DOCUMENT NUMBER: 126:340437
TITLE: Cloning of poly(3-hydroxybutyrate) depolymerase from a marine bacterium, Alcaligenes faecalis AE122, and characterization of its gene product
AUTHOR(S): Kita, Keiko; Mashiba, Shun-ichiro; Nagita, Masatoshi; Ishimaru, Kaori; Okamoto, Kenji; Yanase, Hideshi; Kato, Nobuo
CORPORATE SOURCE: Department of Biotechnology, Tottori University, 4-101 Koyama, Tottori, 680, Japan
SOURCE: Biochim. Biophys. Acta (1997), 1352(1), 113-122
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A DNA fragment that carries the gene coding for poly(3-hydroxybutyrate) (PHB) depolymerase was cloned from the chromosomal DNA of Alcaligenes faecalis AE122 isolated from seawater. The open reading frame encoding the precursor of the PHB depolymerase was 1905 base pairs (bp) long, corresponding to a protein of 635 amino acid residues (Mr = 65208). The promoter site, which could be recognized by Escherichia coli RNA polymerase, was upstream from the gene, and the sequence adhering to the ribosome-binding sequence was found in front of the gene. The deduced amino acid sequence agreed with the N-terminal amino acid sequence of the purified PHB depolymerase from amino acid 28 onwards. Anal. of the deduced amino acid sequence revealed the domain structure of the protein; a signal peptide of 27 amino acids long was followed by a catalytic domain of about 400 amino acids, a fibronectin type III module sequence, and a putative substrate binding domain. The mol. mass (62526) of the mature protein deduced from the nucleotide sequence was significantly lower than the value (95 kDa) estd. on SDS-PAGE, but coincided well with the value (62426) estd. from matrix-assisted laser desorption ionization mass spectra. By comparison of the primary structure with those of other PHB depolymerases, the substrate binding domain was found to consist of two domains, PHB-specific and poly(3-hydroxyvalerate)-specific ones, connected by a **linker** region. The PHB depolymerase gene was expressed in Escherichia coli under the control of the tac promoter. The enzyme expressed in E. coli was purified from culture broth and showed the same catalytic properties as the enzyme from A. faecalis.